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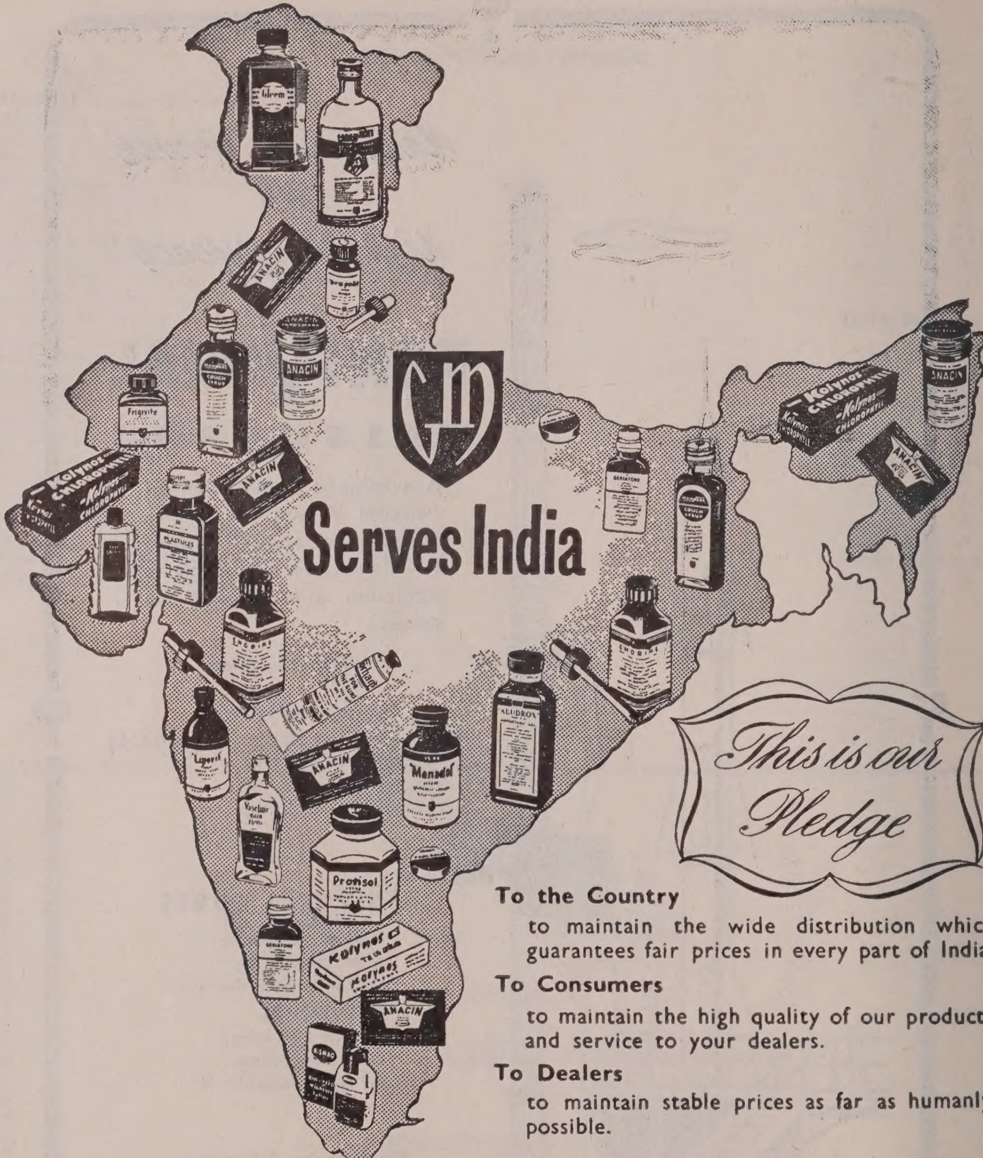
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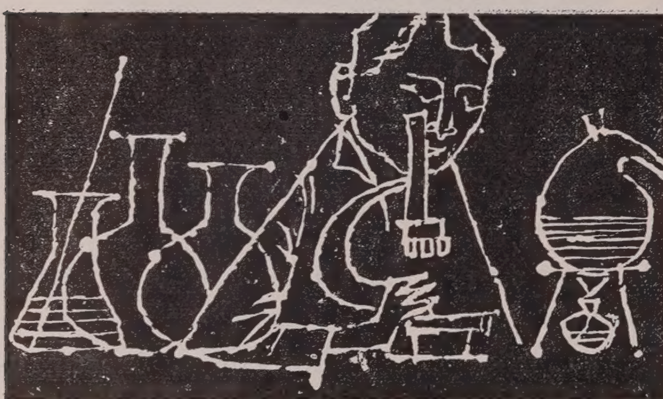

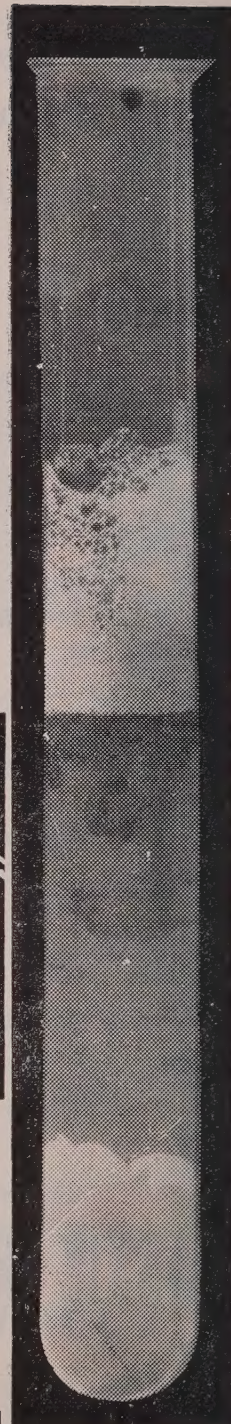

Synergism in Antibiotic Action

BASED upon the mode of action of the antibiotic combinations, Jawetz and Gunnison recognised the synergistic groups as those combinations in which the inhibitory effect was more than the sum total of the individual components. In synergistic combinations as low as 1/3 to 1/20th of the minimum inhibitory concentrations of the antibiotics in normal dosage can give the same effect. Synergism provides an excellent tool for planned chemotherapy. It potentiates antibiotic activity without the concomitant increase in toxicity.

Synergistic action is evident when the proper combination of antibiotics are present simultaneously and not used consecutively. The antimicrobial spectra of the two antibiotics should be similar to some extent. Various techniques for the detection of synergistic combinations have been described. The simplest of these is by placing subinhibitory levels of the antibiotics in assay cups or paper strips in adjacent position on agar plates seeded with the test organism. Synergistic combinations may be detected by the size of zones of inhibitions.

Earlier studies on synergism in antibiotics were merely descriptive, and among the large groups of antibiotics available, various combinations were tested at random. Often, no clear cut differences were distinguished between synergistic and additive combinations. Only recently, with a little more knowledge gained about the mode of action of antibiotics in few cases, it has become possible to plan experiments to obtain synergistic combinations instead of awaiting chance discoveries.

The phenomenon of reversal of antibiotic effect by certain metabolites is well known. Substances like cytosine, L-phenylalanine and even yeast autolysate reverse the inhibition of *Aerobacter aerogenes* by streptomycin. Similarly riboflavin reverses the activity of chlortetracycline in threshold concentrations. As the quantities of these metabolites increase in the medium, the antibiotic activity is proportionately reduced. Inversely, decrease in the quantities of such metabolites would enhance the inhibitory effect of the antibiotic. It is well known that the antimetabolites can inhibit the production of such metabolites. 1:2-Dichloro-4:5-diaminobenzene for instance, can act as an antimetabolite for riboflavin production. While it has no antimicrobial activity by itself, it acts synergistically with chlortetracycline. By following these procedures it should be possible to isolate metabolites acting as reversors of the antibiotic activity, and then finding chemical analogues of the metabolites which inhibit their biosynthesis.

In a few cases where the mode of antibiotic action is known, the operation of the synergistic combinations is explained. If an antibiotic blocks one essential metatolic process in a chain of reactions, the multiplication of the organism is successfully inhibited so long as there is no "leakage". However if leakage occurs due to altered metabolic pathway as a result of mutation, or supply of the metabolite whose biosynthesis is blocked by the antibiotic, the organism can grow again. If a second antibiotic inhibits the reaction in the same metabolic pathway at a different point, the chances of leakage through the first metabolic block and acquiring of alternate pathway for the second antibiotic also are very remote. Hence antibiotic combinations which affect the same reaction usually act synergistically.

Penicillin Reactions — Their Prevention and Treatment

B. B. YODH*

AFTER twenty years of widespread and sustained use throughout the world, pencillin retains its pre-eminent position as the most useful drug against infections. Pharmacologically it is the least toxic of the antibiotics. Present world production of pencillin provides for over 250 million courses of treatment annually.[3] Pencillin being weakly antigenic, if used extensively and indiscriminately, is likely to be associated with sensitization reactions. This is now happening because easy availability and pronounced antibiotic action against the common gram positive organisms has led to such extensive use of this powerful drug. Although the percentage of such reported reactions is comparatively small when set against the background of the vast number of persons benefitting from the use of pencillin, it is important that those using it should be well aware of the possible dangers and the precautions necessary to keep the risks to a minimum.

It is estimated that between 5 to 10 per cent of the population in certain countries is now allergic to pencillin.[9] World's present yearly consumption of about 500 tons of this drug[3] would inevitably cause a certain number of anaphylactic casualties each year, and considering that the age group 19 to 50 is most exposed to treatment and that many people get repeated courses, the incidence of death may be somewhere near 0.4 to 1.2 per million injections. Injected penicillin may be expected to cause some sort of reactions in about 2.5 per cent of the total number of children. In adults, however, the per centage may be about 5 in cases without any previous history of allergy and about 15 among cases with a history of some kind of allergy.[9] Each successive injection tends to increase the incidence of allergic reactions.

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The allergic response, as a rule, is to the pencillin molecule itself or to an intermediate product and not to the procaine or other moiety of the preparation.[3]

Although the injection method of administration is associated with a larger number of sensitized individuals than any of the other methods it would not be correct to conclude that non-parenteral administration of pencillin would not produce allergic reactions.[3]

The fact that a person had not reacted to penicillin treatment earlier is no guarantee that anaphylactic reactions would not occur if he is subsequently exposed to the drug.[3]

In case of pre-established allergy or sensitivity to penicillin the use of the drug should be avoided.[3, 16]

Massive doses of the repository forms of pencillin like procaine penicillin may give rise to a continuous release of the antigen for several days or weeks thus leading to more frequent sensitization and making further treatment with penicillin hazardous.[9]

Types of Allergic Reactions to Penicillin[1—3, 9, 12, 16]

Immediate Reactions: These are rare and are characterized by a rapid onset within a

few seconds to 2 hours of penicillin administration. Manifestations are often severe with anaphylactoid shock, pruritus, urticaria or hives, angioedema, pulmonary oedema and asthma. This type of reaction may result in collapse and death. The reaction usually occurs in patients with previous sensitization to penicillin.

Accelerated Reactions: These are not very frequent. The reactions occur in 12 to 48 hours with intense urticaria, fever, serum sickness—like manifestations and angioedema, and may persist for several days.

Delayed Reactions: This is the most common allergic reaction to penicillin. Symptoms appear in 5 to 21 days, resemble serum sickness and are characterized by skin eruption, fever, urticaria and pain in the joints. The antibody titre slowly rises and reacts with traces of penicillin in the system.

Erythematovesicular or "Id-like" Eruptions: These occur frequently, the onset time extending from a few hours to 3 or even 6 days after administration, and are characterized by eczematous rash particularly on the hands, feet and groin. These reactions are probably a result of an aggravation of a preinduced local tissue sensitivity caused by a superficial fungus infection by an allied mould.

Contact Dermatitis: This occurs frequently in the easily sensitized individuals using topical penicillin preparations such as ointment, and those exposed to the drug in their daily routines as in the case of nurses, pharmacists and doctors and workers in a penicillin factory. The incubation period is 5 to 21 days. In the highly susceptible persons, sufficient absorption of penicillin through the skin may incite any of the more severe types of allergic reactions mentioned above.

Hyperergic Reaction: This is an uncommon type, more severe than the delayed reactions with manifestations of exfoliative dermatitis, anaphylactoid purpura and bullous eruptions. The incubation period ranges from 12 hours to several days.

A related problem is the development of microbial resistance to penicillin particularly in the case of *Staphylococci*. As a result, super-infection can occur in a patient being treated with the antibiotic, or cross-infection in other patients by resistant *Staphylococci* in the same ward.[3]

Sensitization to Penicillin[3, 16]

Anaphylactic shock which may result in fatalities, occurs only in those persons with a history of allergy. Sensitization to penicillin may result from use of the antibiotic as injection, eye or ear drops, lozenges, ointments, inhalation and frequent contact with the drug as mentioned above. In rare instances even the *Penicillium* mould which is always present in air, may get into the body through food or by inhalation and thus act as antigen causing sensitization. It may be noted that very small amounts of penicillin, as may happen in the contact cases, can produce sensitization.

Any of the comon types of penicillins and penicillin preparations in use can cause allergy. Procaine penicillin and procaine penicillin in 2 per cent aluminium monostearate (PAM) have been associated with a large number of reactions because of their widespread use in recent years and because being depot treatments sensitization is more likely to occur. It has, however, been established that allergic response is to the penicillin molecule and not to the procaine part of the compound.

It is the first dose of penicillin following a previous course which may cause immediate anaphylactic reaction. It is generally advised that in treatment-courses, high dosage with short intervals between administrations are preferable, so that the required total dosages can be obtained in a relatively short period. On the other

hand it should be pointed out that prolonged prophylactic or therapeutic courses have been continued in many cases with regular intervals for several years without apparent ill effects.

Personal Factors[3, 16]

Knowledge concerning the effect of individual factors on the anaphylactic reaction is far from complete. It is clear, however, that penicillin sensitized persons with a history of constitutional allergic susceptibility are more prone to and therefore likely to react more severely on subsequent exposure to penicillin than non-sensitized persons. Such allergic states include, asthma, hay fever, rhinitis and pollen allergy.

Contact dermatitis has been frequently noticed in the history of anaphylactic cases. Eczematous reactions of the erythematovesicular type and delayed serum-sickness like urticarial reactions due to penicillin are not, however, precursors of anaphylactic reactions. Even such mild signs as flushing of face, itching, a tingling in the tongue or fingers, a peculiar taste in the mouth, dizziness and fever, are indicative of sensitivity which may lead to fatal anaphylactoid shock on re-exposure to penicillin. Sensitivity of the anaphylactic type may be transient, but there is no guarantee that a person who showed no reaction to penicillin at one time may not experience anaphylaxis or any other reaction on a subsequent administration of the drug.

As regards time factor, the onset of anaphylactic reactions may be sudden or immediate within a few seconds and of a violent nature on administration of penicillin. In the case of delayed and hyperergic type reactions, the onset may occur from a few hours to several days.

Treatment and Precautionary Measures[3, 9]

The first requisite is preparedness for any emergency. Tourniquet, sterile syringes, and prescribed drugs for shock and for other reactions should be available at hand.

Before administering penicillin the patient should be questioned carefully regarding previous exposure to the drug and any manifestations of allergic conditions in himself or his family. It has been suggested that persons who have shown sensitivity to penicillin may be asked to carry cards indicating the case history for reference. Even mild previous reactions are a contra-indication to re-exposure to penicillin. Administration of penicillin should be particularly avoided in cases with a history of asthma, hay fever and other allergic diseases. As an additional precaution a skin test may be done, but the patch test is of value in contact dermatitis only. On the other hand immediate intradermal test, although positive in cases of anaphylaxis, may itself produce severe anaphylactic reactions. Further, a negative skin test does not necessarily preclude sensitivity to penicillin.

Penicillin should be injected slowly by intramuscular route and the patient watched during the injection for any sign of reactions. The patient should remain in the consulting room at least for 15 minutes after the injection. Should anaphylactic symptoms appear, adrenaline is to be given subcutaneously or intravenously or even directly into the heart in extreme shock. Administration of antihistaminics like hydrabamine hydrochloride or tripelennamine is also useful. In case of respiratory distress, injection of aminophyllin is recommended. For prolonged depression of blood pressure, continuous drip infusion of *l*-arterenol or plasma may be helpful. In protracted cases intravenous hydro-cortisone succinate or intramuscular ACTH is recommended. Penicillinase, a penicillin splitting enzyme, administered 800,000 units intravenously followed by 800,000 units intramuscularly, has proved effective for arresting anaphylactic shock. If this is done, another antibiotic should be given at the same

time for the infection. It must also be noted that systemically administered enzymes may in themselves produce allergic reactions.

The best means of reducing the abuse of penicillin and of avoiding unnecessary exposure of the population to sensitization and development of resistant strains of micro-organisms, is through education of the public and medical men on these aspects of antibiotic therapy. There must be clear and sound medical indications for use of penicillin. It should not be used in minor ailments like common colds, eczema, impetigo, pharyngitis, bronchitis, etc. Its topical use should not be encouraged, and the drug should have no place in tooth pastes, chewing gums and cosmetics. Because of its easy availability and wide usefulness, use of penicillin should not replace sound diagnosis. Above all, self-medication should be strictly avoided even by medical men and nurses.

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Price Policy*

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THE price of a commodity, according to the old principles of economics, was defined as the amount of money which it will command. A commodity which sold for a high price had a high value in terms of accepted medium of exchange and this price indicated and measured the values of goods in terms of a commonly accepted medium of exchange. This old orthodox definition has undergone radical changes with changes in society, economic values and in a period of planned economy. Similarly, it was stated that the price is the result of the interaction of demand and supply. If the demand is more than the supply the price will rise and if it is less the price will fall. While this principle may be largely true in a free economy, it does not always operate in case of essential commodities where the social needs and government policy take certain steps to check undue rise in prices of commodities which are considered vital for the building up of the national economy. The question of price policy, therefore, has to be examined in the light of the modern concept of a society which has accepted planned production and distribution in order that the consumer gets goods and services at as low a price as possible with due regard to the reasonable interests of the producer also. The price has, therefore, a great deal to do with the nature of the commodity, *i.e.*, the degree of its usefulness to the society, its cost of production, reasonable margin of overheads and profits, and what the traffic can bear.

2. In order to determine what exactly goes into the cost of production and what is the cost of production of each given item at each particular stage of production, a number of things have to be considered. The normal items going into the cost of production and of sale of an article are costs of raw materials, power, processing, labour, overheads, depreciation of plant and equipment and obsolescence, cost of distribution including salesmanship and advertisement, reasonable return on the capital invested, etc. Cost accounting is a continuous process and the price-fixer has to be equipped with figures of cost of production at different stages of processing and also of the finished article. It is the function of the cost accountant to provide the price-fixer with the best possible information that will assist him to apply the marginal price-fixing technique. In order to maintain the cost of production at as low a level as possible, the efficiency of the plant and equipment has to be maintained at the highest level possible. This would mean that raw materials would have to be purchased at the cheapest price possible at the most advantageous time for purchase of such materials. The stocks of raw materials have to be maintained at such levels that while large amounts are not unnecessarily locked up in store purchases, low stocks, on the other hand, should not jeopardise operations. Care has also to be taken to see that the maximum per capita output is obtained from the labour employed on that job. Automation has its obvious advantages as a labour saving device; but in a country like India, a golden medium has to be

* Paper presented to the Seminar on Management of Public Industrial Enterprises, sponsored jointly by the Government of India and the United Nations, New Delhi, 1-11 December 1959.

struck between automatic machines and employment potential, because industrialisation while increasing the national wealth, has also to play an important role in providing jobs and opportunities of employment to the growing millions of the country. As the aim of our Constitution is to provide equal opportunities and better standards of living to the people of this country, the public sector factories have a special responsibility in this matter not only to provide as many jobs as possible but also to set a pattern for efficiency and labour-welfare for other industries in the private sector. It is for this reason that amenities like housing, free education, free medical aid to the workers and their families, sufficient leave and opportunities for rest and recreation and other similar amenities are provided in a large measure by the public sector factories. This policy naturally involves greater expenditure and some of it might appear unproductive in as much as it cannot be directly connected with any particular plant, equipment or process of manufacture. It, however, gives a very valuable, though not tangible, return in the shape of greater satisfaction of the worker and hence improved efficiency. All these items of amenities naturally go into the cost of production and it is therefore, necessary to ensure that the workers who enjoy these benefits of relatively higher wages and amenities, also give better and higher output. This would depend largely on the realisation on the part of the workers and the extent of co-operation that they and their unions are able to extend to the management.

3. While talking of efficiency of production, considerable attention has also to be devoted to the quality of products. For this purpose standards have to be laid down and rigidly enforced under a system of strict inspection. In this direction, the Indian Standards Institution has been doing extremely useful work. Various testing stations set up by the Government as well as by other agencies have also been playing an important role. What is however required is an extremely competent independent and strict inspection unit in each manufacturing concern; and no article should be allowed to leave the factory unless it has been severely tested and passes all the tests.

It is not easy for a manufacturer to resist the temptation of pushing his goods into the market as rapidly as he can make them. Such temptations would be all the greater if the commodity is in considerable demand. The lure of quick sales and early realisation of cash coupled with the desire to fulfil the demands as early as possible, very often lead the manufacturer to close his eyes to the vital need of strict inspection and to go ahead with the despatch of goods even at the risk of deterioration of standards. The manufacturer who cares for his own reputation and that of his products, must resist these temptations and reject any article which does not come up to the requisite standards. In the pharmaceutical industry, and more particularly in the case of a life-saving drug like penicillin, such a step is absolutely essential.

4. Once efficiency and quality of production have been assured, the next question having a bearing on the price is that of distribution including sales, sales promotion and publicity and the margin for distribution cost to be included in the price. This varies from industry to industry. In a heavy engineering industry producing a few units, the margin per unit would naturally have to be higher. In a light consumer industry producing hundreds of thousands of units of consumer goods, the margin might be smaller because the overall profits on a large turnover will be more. There are however certain consumer goods industries where the margin has got to be fairly high due to the very nature of the activities involved. An outstanding example of such an industry is the pharmaceutical industry. There may perhaps be an impression in the public mind that the pharmaceutical industry charges exorbitant prices for its products

and thus indulges in profiteering. This impression perhaps ignores the fact that such industries, in order to remain in the market, have got to maintain extremely efficient and expensive research and development units. The invention of a new medicine or drug would necessarily involve a long and patient period of research and large expenditure. Out of a hundred or more researches on hand, perhaps one might prove successful and even that successful research might not be found commercially profitable as a result of some other manufacturer obtaining better results or having succeeded in publicising his products better. As drugs go out of vogue, and diseases develop resistance to a particular drug, new drugs and remedies superior to the previous ones have got to be found. Moreover, a manufacturing process has to be evolved and adopted by a system of trial and error and a lot of work in the laboratory. The pilot plant operations in the initial stages have to be done for obtaining reasonable yields from new raw materials. Once mass production by rational methods starts, the price of these drugs decreases considerably. Penicillin, is an outstanding example of such a process. When it was first made, it cost a fortune and its storage under refrigerated conditions was a problem. Now it sells as cheap as about 50 nP. for an average vial. When the present Penicillin Factory at Pimpri was planned, the cost of production was estimated at Rs. 1.25 per mega unit in bulk which was comparable to the international price prevailing at that time. Since then the technique of penicillin production has been so much revolutionized as a result of further research that it has been possible to obtain much higher yields during the process of manufacture. This has naturally resulted in substantial reduction both in the internal and international price of this drug. The same penicillin is at present selling in bottled form at an approximate price of about 50 nP. for an average packing which would mean that the cost of production would be still less. Similarly, its bulk price in the international market has gone down to less than Rs. 0.25 per mega unit. This would illustrate the point that once production stabilises in case of an important drug in the pharmaceutical industry and it has good prospects of holding the market for a reasonably long time, the price is bound to fall substantially.

In addition to the expenditure on research, provision has to be made in certain industries for obsolescence of the plant and machinery. For example, if a new and more potent drug is discovered or if a biological product is economically synthesised, substantial changes in plant or even replacement of the entire plant may have to be carried out resulting in the investment being rendered partly or wholly infructuous. This is happening almost every day in many industries and the problem has to be faced and tackled as and when it arises. Every such manufacturer, therefore, has to lay aside a substantial amount to provide for this contingency and to use it as and when necessary.

5. A third factor which has to be provided for the calculation of the price, is the expansion programme for the plant and equipment. As the public demand for the products grows, factories have to expand their production not only in the particular branch of production in which they are engaged but they have also to take up other products of an allied nature which could also be produced economically and thereby benefit the industry as well as the public. Funds for such expansion and production of allied products can be found either by increasing the share capital, borrowing funds, by issuing debentures or taking medium or long term loans or by utilising the reserves of the existing plant. The first system of floating further share capital would increase the burden on the factory for a long period and would also make an inroad on the capital available in the market,

which in an expanding economy of a country like ours, is not always adequate. The second system of floating debentures or taking loans would also involve payment of interest and an added obligation on the factory. The rates of interest for these loans would naturally be higher than those at which these lending institutions themselves would be borrowing. It is, therefore, advisable to build up general reserves for capital expansion of the project and to utilize these reserves of the factory itself for its future development. The national policy of taxation should also be framed in such a way that it would encourage production units to accumulate larger and larger reserves for development and to distribute only such amounts as dividends as would be commensurate with the normal returns on capital investment in the country and thereby help capital formation for the future expansion of the country. Any factory therefore including in its price a reasonable margin for future expansion without unduly upsetting the price structure of the country, should be considered to have taken a step in the right direction and be encouraged to do so. It should not be difficult to ensure that the funds earmarked for expansion and development, are utilized for the purpose only. The Company Law Administration or Income Tax Administration or the Ministry of Commerce and Industry in the Development Wing, would be the proper agencies to ensure correct utilization of such funds. Provision in the price structure for future development is all the more necessary in a public sector factory where the required funds naturally come from the taxpayers' pockets. If adequate funds are not available with the factory, Government has to find them by resorting to further taxation.

6. While it is extremely difficult to lay down hard and fast rules for a progressive price policy in a country like India in relation to the various goods and services produced by the various concerns, certain fundamental principles and policies can be of guidance both to the Government and to the industry for evolution of a proper price policy.

(a) Once the cost of production is brought down as low as possible as a result of increased efficiency and production and without adversely affecting the standards and quality of an article, a substantial portion of these benefits should be passed on to the consumer.

(b) Certain portion of the benefits of this increased efficiency and lower cost, should also go towards increasing provision for obsolescence and depreciation of plant, welfare activities for the workers and future development.

(c) Assessment of cost of production being a continuous process, revision of prices at suitable intervals also becomes a normal activity of a progressive industrial undertaking. In working out these price reductions, the Board of Directors or the policy-making body has to consider carefully the incidence of various other factors even after a scientific cost accounting is done.

(d) After having carefully fixed the price based on the above principles, the announcement of the price reduction should not be made at frequent intervals or too early before the effective date. At the same time reasonable time has to be given to the public and to the distribution agencies for adjusting their sales to the revised situation. Too many and too frequent changes, even if the prices are to be reduced, instead of being appreciated by the public are likely to shake their conafidence in the price structure and might adversely affect sales.

(e) Widest possible publicity has to be given to the revised prices in order that the information reaches the con-

sumer in every part of the country. A debatable point in this connection is whether it is possible for all producers to fix one or more consumer prices for the country as a whole and to ensure that every consumer even in the remotest part of the country will get the product at that price. This is indeed a very tall order especially in a vast country like India, where channels of transport and communications are not always developed in equal proportions. If local shortages develop, the economic principle of demand and supply would start operating in spite of the fixation of a consumer price. Moreover, while a producer may have certain influence and control over the dealer or the wholesaler at certain points of his distribution pipeline, it is not always possible either for the distributor or the producer to force the retailer to charge a particular price to the consumer. Even if such an agreement is taken from the retailer, it would not be worth the paper on which it is written. In case, therefore, of commodities considered most essential for the life of the community, nothing short of an ordinance or law like the Essential Commodities Act would achieve the object of price control. Price stabilisation, however, can be achieved by fixing a trade price and maintaining adequate supplies.

7. Summing up the position, Government of India, which aims at a socialist pattern of society, has adopted a policy of progressively bringing down the prices of goods and services wherever possible and they are able to do so with greater speed in the public sector factories over which they have a direct control. This enlightened policy is sought to be implemented by encouraging production, maintenance and improvement of high standards of quality of the goods, permitting adequate provision for depreciation and development of the industries, promotion of increased labour welfare activities, permitting reasonable return on the capital investment and passing on the benefit of the increased efficiency and lower cost of production to the consumer.

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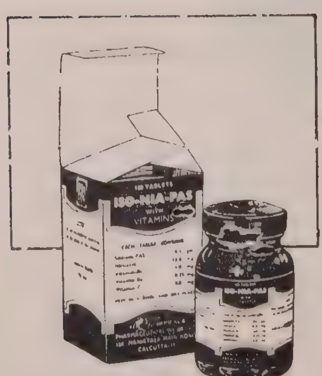

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S. S. VAIDYA,
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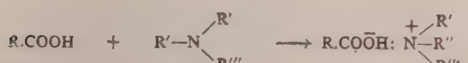
FREE penicillin being an acid forms quarternary ammonium compounds with amines which are organic bases. The penicillin salt formation can be represented as follows :



Benzylpenicillin acid — Amine — Penicillin salt

where R is penicillin. R'. R'' and R' '' are alkyl, alkylene, alicyclic, aralkyl or heterocyclic groups. Majority of the amine salts of penicillin have low solubility in water and, therefore, give prolonged blood levels of the antibiotic. Some of those with low toxicity have proved to be therapeutically useful *e.g.* benzathine penicillin. Anaesthetic and specific tuberculostatic actions have been observed with aminosalicylate salts. Some of the substituted alkylenediamine salts show anthihistaminic as well as repository action. In addition to thereapeutic uses, efficient recovery of penicillin from the rich acetate can be affected via the preparation of salts like that of *N*-ethylpiperidine.

The amines which have been used in penicillin salt preparation are listed in the appended table. The amines are classified into aliphatic, alicyclic-substituted aliphatic, benzylsubstituted, aralkyl, aromatic esters, amino acid esters, heterocyclic, sulphonamides, alkaloidal, steroidal, rosin and miscellaneous amines. The amides are listed at the end of the table. Much of the literature is in the nature of patents and the references are, therefore, mostly to *Chemical Asbstract*, which has been checked from v. 44 (1950) through v. 51 (1957), and the names are given as reported therein. Brief notes on the pharmacological and other properties of the penicillin salts are given in the table wherever such information was available.

Penicillin salts of aliphatic, alicyclic-substituted aliphatic and alicyclic amines were mostly reported prior to 1952. A series of penicillin salts of the derivatives of ethylenediamine reported from this laboratory show some interesting properties. Solubility of the salts in water decreases with the increase in the length of alkyl chain attached to the two N atoms of ethylenediamine ; the bitter taste of the salts decreases with increase in the alkyl chain length and as the solubility decreases. *N*, *N*'-di-*n*-propyl - and *N*, *N*'-di-*n*-octyl-ethylenediamine salts maintain effective blood levels of penicillin for 5 or more days when injected intramuscularly in rabbits. The latter salt also shows powerful and prolonged antihistaminic activity.[104]

There are few benzyl-substituted alkylenediamine salts of penicillin reported although, on an analogy with dibenzylamine and benzathine penicillins, some of them may prove therapeutically useful.

Since 1950 a large number of penicillin salts of aralkylamines have been reported and a great proportion of them have low solubility. repository action, relatively low toxicity and, therefore, suggested for therapeutic use.

Procaine penicillin representing the group of amines with aromatic acid substitution, was the first successful repository form of the antibiotic and is in wide use even to-day. Several other salts of this type of amines have also been reported to have repository action.

Although there are a large number of penicillin salts with heterocyclic compounds few of them have attained thereapeutic importance. The *N*-ethylpiperidine salt is used for the recovery of penicillin from the rich acetate.

Among the few alkaloidal penicillin salts, quinine and emetine salts have shown some therapeutic value giving prolonged effective blood levels of penicillin.

Steroidal amine salts, comparatively few of which have been reported, have low solubility and show repository action. Three new steroidal amine salts in which *N*-benzyl compounds of cholesterol were used have recently been reported[7] from this laboratory. In preliminary studies on serum concentrations in rabbits given 100,000 units intramuscularly, *N*-benzyl-6β-amino-3:5-cyclocholestane salt produced an intermittent rise and fall in the therapeutic level of penicillin. Whether this behaviour is due to the presence of the 3-5 bond in the steroid nucleus or merely because of the attachment of the benzyl amine group at the 6-position is yet to be determined. From the blood level curves of *N*-benzyl-3β-cholesterylamine and *N*-(cyclohexylmethyl)cholestan-3β-ylamine salts, it appears that the hydrogenation of the parent base of the former salt to the completely reduced base of the latter salt extends the average therapeutic serum level of penicillin to almost 10 days, that is about twice the period for the salt of the unreduced base. Further, the average curve of the latter salt dips down at 48 hours and rises again to taper out gradually in time. Here again it is to be studied if it is the reduction of the 5-6 double bond of the steroid moiety or the reduction of the phenyl portion which leads to the different behaviour of the salts.

Hydrabamine penicillin of the rosin amine group has therapeutic value comparable to benzathine penicillin. Penicillin salts containing mixtures of certain rosin amines have been used in antiseptic preparations such as impregnated bandages, ointments, etc.

In the Miscellaneous group of amines are included biguanide, urea and urethan derivatives and some amines of different general formulae with different substituents of the alkyl, alkenyl, alicyclic, alicyclic-alkyl, aralkyl, aromatic and heterocyclic groups attached. Most of the penicillin salts of this group are sparingly soluble and, therefore, show depot action. Salts of substituted alkylenediamines and those with the general formula $XCH(R)CH_2$-NR_2 have antihistaminic action in addition to antibacterial activity.

Of the large number of amine salts of penicillin very few have therapeutic importance. This may be due to several reasons : Undesirable side effects, poor stability, blood level of the antibiotic not being better or superior to that of salts already in use, or the cost of synthesis of the amines and their large scale production may be uneconomical.

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Amine	Remarks on the Penicillin Salt	Ref.
I. Aliphatic Amines		
Trimethylamine		82
Ethylamine		31
Triethylamine		82
3, 4-EtO(HO) $C_6H_3CH_2NHCH_2$-CH_2NHCH_2Ph	Therapeutic level of penicillin in blood extends to 48 hr. or more	5
N, *N*'-dibenzylethylenediamine	Therapeutically successful repository penicillin salt	98
N, *N*'-bis (1-naphthylmethyl)-ethylenediamine	Injection of a suspension in aqueous or oily medium gives prolonged blood level of penicillin	86
N, *N*'-bis (2-furfurylidene)-1, 2-diaminoethane		99
N, *N*'-difurfuryl-1, 2-diaminoethane		99
N, *N*'-bis(α-phenylethyl)ethylene-diamine		95
N, *N*-dimethyl-*N*'-benzyl-*N*'-(2-pyridyl)-ethylenediamine		41
N-pyridyl-*N*-chlorobenzyl-*N*, *N*'-dimethylethylenediamine		36
2-[(*p*-Methoxybenzyl) (2-dimethyl-aminoethyl)-amino]-pyridine		87
iso Propylamine		31, 32
(1, 1-Dimethyl-2-hydroxypropyl) amine	Low toxicity; repository action when given orally or by injection	108
dl-$MeEtCHNH_2$		32
3-Amino-1, 1, 1 trichloro-2-propanol	Sparingly soluble, gives extended blood level of penicillin	81
$(Me_2CH)_2NH$		32
Tripropylamine		82
Tri*iso*propylamine		82
Butylamine		31
(1 and 2)-Methylpentyl amine	Compares with procaine penicillin with respect to persistency and uniformity of blood levels and toxicity	109
(1-Methyl*iso*pentyl) amine		109
2-Amino-4 methyl hexane		75
N,*N*-dimethyl-2-ethylhexylamine	Salts of higher tertiary aliphatic amines give prolonged therapeutic blood levels of penicillin when administered parenterally	73
Hexyl *N*,*N*-dimethylhexylamine		73
2-Aminoheptane		75
dl-$MeAmCHNH_2$		32
2-Amino-6-methylheptane		60

Amine	Remarks on the Penicillin Salt	Ref.
N, N-dimethyloctylamine	Salts of higher tertiary aliphatic amines produce prolonged therapeutic blood levels of penicillin when administered parenterally	73
N, N-diethyloctylamine		73
N-methyl *N*-propyloctylamine		73
Tertoctylamine		33
Nonylamine		89
N, N-dimethylnonylamine	Salts of higher tertiary aliphatic amines produce prolonged therapeutic blood levels of penicillin when administered parenterally	73
N-ethyl-*N*-methylnonylamine		73
N, N-dimethyldecylamine		73
N, N-dimethylhendecylamine		73
N, N-dimethyldodecylamine		73
N, N-dipropyldodecylamine		73
N, N-dimethylpentadecylamine		73
N, N-dimethyloctadecylamine		73
II. Alicyclic Amines		
Cyclopropylamine		53
Cyclobutylamine		53
Cyclopentylamine		53
Cyclohexylamine		31,53
2-Methyl-cyclohexylamine		31
4-Cyclohexylcyclohexylamine		19
Dicyclohexylamine		32
dl-1, 2, 3, 4 Tetrahydro-2-napthylamine		32
Bornylamine		54
III. Alicyclic Substituted Aliphatic Amines		
1-Cyclopentyl-2-(methylamino)-propane		75
1-Cyclohexyl-2-(methylamino)-propane		75
IV. Aromatic Amines		
p-Aminobiphenyl	Repository Action	36
N-(2-aminoethyl) anthranilic acid derivatives	Sparingly soluble, give prolonged blood levels of penicillin	81
V. Aromatic Substituted Alkylamines		
Benzylamine		100
(α-Benzoylbenzyl)-*N*-methylamine	Suggested for use in patients allergic to procaine penicillin	46
α-Methyl-2-naphthalene-methylamine	Sparingly soluble, gives prolonged blood levels of penicillin	81
Aminodiphenylmethane		60

Amine	Remarks on the Penicillin Salt	Ref.
4, 4'-Dimethylbenzhydrylamine	Effective blood levels of penicillin when given orally or intramuscularly to dogs	33
Dichlorobenzhydrylamine		33
N-methylbenzhydrylamine		33
N-(*p*-methoxybenzyl) benzhydryl-amine		33
4-Dimethylaminobenzhydrylamine		33
N, *N*'-di (*p*-methoxybenzyl)-*p*-xylenediamine		33
N, *N*'-dibenzyl-*p*-xylenediamine	Effective blood levels of penicillin when administered orally or intramuscularly to dogs	33
$PhCH_2CH_2NH_2$		18, 100
N-benzyl-β-phenylethylamine	Reported better than procaine penicillin. Low solubility, toxicity, does not cause irritation, and is stable in aqueous solution	51, 70
N-diphenylmethylphenethylamine		6
Diphenylethylamine		93
$C_6H_5C_6H_5CHCH_2NH_2$		27
p-$Me_2NC_6H_4CH{:}NHCHMeCH_2Ph$		72
N-methyl-1, 2-diphenyl-2-hydroxy-ethylamine	Decreased the incidence and severity of allergic reactions in patients in whom such reactions were observed with other salts of penicillin. A single 3 lac units dose gave effective penicillin blood level for about 18 hr.	61
Methyl [1, 2-bis (*p*-methoxyphenyl)-2-hydroxyethyl] amine	Sparingly soluble, repository action	107
α-Phenyl-β-(cyclohexylamino) ethyl-chloride		13
$PhCH_2CH(NH_2)Me$	Therapeutically useful	100
Diphenylethanolamine		106
α-Phenylethylenediamine		8
$PhCH(NH_2)CH_2NHEt$		8
$PhCH(NHEt)CH_2NHEt$		8
$PhCH(NH_2)CH(NH_2)Ph$		8
N,*N*'-di (2-furoyl)-α, α'-diphenyl-ethylenediamine		8
N, *N*'-difurfuryl-α, α'-diphenyl-ethylenediamine		8
$PhCH(NHMe)CH(NHMe)Ph$		8
α, α-Diphenyl-*N*, *N*'-di (2-heptyl) ethylenediamine		8

Amine	Remarks on the Penicillin Salt	Ref.
N, N-di (*n*-decyl) ∝, ∝'-diphenyl-ethylenediamine	Therapeutically useful	8
N, N'-diphenyl-∝, ∝'-diphenyl-ethylenediamine		8
N, N'-dibenzyl-∝, ∝'-diphenyl-ethylenediamine		8
N, N'-diethyl-∝, ∝'-di (*p*-hydroxy-phenyl) ethylenediamine		8
N, N'-diethyl-∝, ∝'-di (*p*-methoxy-phenyl) ethylenediamine		8
N, N'-dimethyl-∝, ∝'-di-benzyl-ethylenediamine		8
dl-Me ($PhCH_2$) $CHNH_2$		32
1-Phenyl-2-aminopropane	Useful in isolation and purification of penicillin	17
dl-Me ($PhCH_2CH_2$) $CHNH_2$		32
$C_6H_5C_6H_{11}$ CH (CH_2) $_2NH_2$		27
1-Phenyl-1-hydroxy-2-aminopropane		17
dl-Me (4-$MeC_6H_4CH_2$) $CHNH_2$		32
dl-1-(3, 4-Dimethylphenyl)-2-aminopropane		32
dl-Me (2, 4-$Me_2C_6H_3CH_2$) $CHNH_2$		32
dl-Me (3, 4-$Cl_2C_6H_3CH_2$) $CHNH_2$		32
dl-Me (2, 4-$Cl_2C_6H_3CH_2$) $CHNH_2$		32
dl-1-(5, 6, 7, 8-Tetrahydro-2-napthyl)-2-aminopropane		32
2-Benzyl-2-aminopropane		33
1-Dimethylamino-3-acetoxy-3, 3-dibenzylpropane	Sparingly soluble, repository action	78
2-Benzyl-2-benzylaminopropane		33
2-Benzyl-2 (*p*-methoxybenzylamino) -propane		33
Benzhydryldi*iso*propylamine	Repository action. Useful in cattle feeding	3
$C_6H_5C_6H_5CH$ (CH_2)$_2$ NEt_2		27
$C_6H_5C_6H_5CH(CH_2)_2$ morpholine		27
d- and *l*-, methyl-1, 2-diphenyl -2-hydroxyethylamine	Clinically tested and of therapeutic value	107
4-Chloro-*N, N*-dimethyl-4-phenyl-butylamine	Sparingly soluble, repository action	81

Amine	Remarks on the Penicillin Salt	Ref.
Oxybenzyl*iso*butylamine		84
dl-Ph-$(CH_2)_4$-$CH(NH_2)$ Me		32
3-(*p*-Methoxyphenyl) -*N*,*N*-dimethyl heptylamine	Low solubility. Parenteral administration of 5000 u/kg. body weight, gives greater concentration for longer periods of penicillin in the animal body than that obtained with procaine penicillin	85
N-*p*-methoxybenzyl-tertoctylamine		33
VI. Amino Derivatives of Ether		
$Ph_2CHOCH_2CH_2NMe_2$		68
2-Benzylphenyl-2-dimethylamino-ethyl ether	Sparingly soluble, repository action	41
Di-(3-benzylaminopropyl) ether		83
o-Benzylphenyl-2-dimethylamino-ethyl ether	Repository action, useful for oral use and external applications	41
$H_2NCMe_2CH_2OR$ (R-*iso*bornyl)	Relatively stable, slightly soluble in water	4
VII. Aliphatic Ester Substituted Amines		
2-Dimethylaminoethyl ester of diphenyl acetic acid	Sparingly soluble, repository action	81
α-Phenyl-β-morpholinoethanol chloroacetate		27
2-Dimethylaminoethyl ester benzillic acid	Sparingly soluble, repository action	81
2-Methyl-l-piperidinepropanol esters		81
3-Morpholinopropyl cinnamate		81
VIII. Alicyclic Ester Substituted Amines		
2-Diethylaminoethyl-l-cyclohexyl-cyclohexane carboxylate		77
IX. Aromatic Ester Substituted Amines		
2-Diethylaminoethyl-*p*-amino-benzoate	First successful repository form of penicillin in extensive therapeutic use	91
2-Diethylaminoethyl-2-methyl-4-aminobenzoate	Solubility lower than that of procaine penicillin	77, 25, 28
2-Diethylaminoethyl-*p*-chloro-4-aminobenzoate		77, 25
2-Dimethylaminoethyl 2, 4-diaminobenzoate		25
2, 4-$(H_2N)_2C_6H_3CO_2(CH_2)_2NEt_2$	Solubility lower than that of procaine penicillin	28
Hydroxyprocaine	*In vitro* tuberculostatic effect like *p*-aminosalicylic acid effect without the penicillin moiety co-operating	72a

Amine	Remarks on the Penicillin Salt	Ref.
2, 3-HO $(H_2N)\ C_6H_3CO_2\ (CH_2)_3$-NH-Bu-*iso*		44
2, 4-HO $(R''' HN)C_6H_3CO_2CH_2CH_2NR' R''$ where R', R″ and R‴ are alkyls contg. not more than 4 C atoms		74
2, 4-HO $(H_2N)C_6H_3CO_2CH_2$-$CH_2NR' R''$ where R′ and R″ are alkyls of 1,3 or 4 C atoms		74
2-Diethylaminoethyl-4-amino-salicylate		43
2, 4-HO $(H_2N)\ C_6H_3CO_2CH_2CH_2$-$NEt_2$ and deriv. N-oxide and N-hydrated oxide	Preparation of this salt applicable for purification of penicillin from impure solutions	74
2, 4-HO $(H_2N)\ C_6H_3CO_2CH_2$-$CH_2NC_5H_{10}$		44
2, 4-HO $(H_2N)\ C_6H_3CO_2CH_2CH_2$-$NCH_2CH_2CH_2CH_2CH_2$ and deriv. N-oxide and N-hydrated oxide		74
2, 4-HO (BuNH) $C_6H_3CO_2CH_2$-CH_2NEt_2		44
2, 4-HO (PrNH) $C_6H_3CO_2CHMe$-$CHMeCH_2NEt_2$		44
Dialkylaminoalkyl *p*-hydroxy-benzoate		62
p-$HOC_6H_4CO_2\ (CH_2)_2N\ (C_2H_5)_2$	Sparingly soluble. Gives, on injection, penicillin blood levels higher than that obtained with procaine penicillin; also shows protracted effect	103,57
2-Diethylaminoethylester of 4-amino-1-naphthoic acid	Sparingly soluble, gives prolonged blood levels of penicillin	77
X. **Amino Acid Esters**		
Ethyl *dl*-phenylalanine		102
Ethyl-*l*-phenylalanine		102
Amyl phenylalanine	Therapeutically useful	102
Ethylester of tyrosine		102
Hexyl tyrosine		102
$PhCH_2CH\ (NH_2)\ CO_2R$ (R-alkyl)	Sparingly soluble	103
XI. **Amino Derivatives of Sulphone**		
p-$NH_2CH_2C_6H_4SO_2Me$		101
p-PhS $(O_2)\ C_6H_4CH_2NHCMe_3$		52
p-PhS $(O_2)\ C_6H_4CH_2NHCMe_2CH_2$-$CMe_3$	Therapeutically useful	52
p-PhS $(O_2)\ C_6H_4CH_2NHCH_2Ph$		52
$(p\text{-}(PhCH_2NHCH_2)\ C_6H_4)_2\ SO_2$		52

Amine	Remarks on the Penicillin Salt	Ref.
XII. Amino Derivatives of Sulphonamides		
∝-Amino-*p*-toluenesulphonamide		58
∝-Amino-*p*-toluenesulphonamide-Schiff bases.		72
p-$NH_2CH_2C_6H_4SO_2NHMe$	Sparingly soluble, therapeutically useful	101
N-methyl-(*p*-aminomethyl) benzene-sulphonamide		48
p-$H_2NCH_2C_6H_4SO_2NHCH_2Ph$		48
p-$NH_2CH_2C_6H_4SO_2NMe_2$	Sparingly soluble, therapeutically useful	101
p-$NH_2CH_2C_6H_4SO_2NEt_2$	Sparingly soluble, therapeutically useful	48,101
XIII. Steroidal Amines		
Cholesterylamine	Sparingly soluble, therapeutically useful	64
Cholesteryl (diethylamino) acetate	Prolonged blood levels of penicillin. Used in wax-vegetable oil medium for injection or in tablet form for oral administration.	16
XIV. Rosin Amines		
Mixture of dehydroabietylamine, dihydroabietylamine and tetrahydroabietylamine	Combines the properties for penicillin and the bactericidal properties of amines. Useful as stable form of penicillin in antiseptic tooth powders, tooth pastes, ointments, impregnated bandages, and in products for veterinary medicine and poultry and livestock feeding.	15
N, *N*'-bis (dehydroabietyl) ethylene-diamine	Hydrabamine penicillin G is comparable to Benzathine penicillin in respect of repository action and toxicity. Clinically useful.	21
N, *N*'-bis (dihydroabietyl) ethylene-diamine		21
N, *N*'-bis (tetrahydroabietyl) ethylenediamine		21
XV. Heterocyclic and Heterocyclic Substituted Compounds		
Tryptamine		18
N-benzylfurfurylamine		33
N-(*p*-*iso*propylbenzyl) furfurylamine		33
N-(5-nitro-2-furfurylidine)-3-amino-5-(1-piperidylmethyl)-2-oxazolidone		30
Phenazone		59
Amidopyrine		59
Histamine		18

Amine	Remarks on the Penicillin Salt	Ref.
4, 5-Diphenylimidazole		47
2-(2-Furyl)-4, 5 diphenylimidazole		47
2, 4, 5-Triphenylimidazole		47
2, 4, 5-Tri (*p*-methoxyphenyl)-imidazole		47
6a, 3a-Diphenyltetrahydro-imidazi-midazole-2, 5 (1, H, 3H)-diimine	Sparingly soluble, non toxic	34
1-(*p*-chlorobenzyl)-2-(pyrrolidyl-methyl) benzimidazole		66
2-Propylimidazoline and derivs.		105a
2-(*N*-phenyl-benzylaminomethyl)-4, 5-dihydroglyoxaline	Oily suspensions give prolonged blood levels of penicillin	87
2, 2-Diphenylimidazolidine		47
2, 2-bis (*p*-chlorophenyl) imidazolidine		47
2-Methyl-4, 5-diphenylimidazolidine		47
1, 3-Dibenzylimidazolidine		47
Aminothiazole		69
2-Aminothiazole		71
2, 4-Dimethylthiazole		71
Pyridine		56
2-Aminopyridine		49
3-(Diethylaminoethyl) pyridine	Sparingly soluble, repository action.	81
2-[-*p*-Chloro- ∝ -(2-dimethylamino-ethyl) benzyl]-pyridine	For oral administration and external application. Repository action	40
2, 6-Diamino-3-phenylazopyridine		42
6-(3-*iso*Propylaminopropylamino)-3-methyl-7H-dibenz-(*fij*)-*iso*-quinoline-2, 7 (3H) dione		23
2, 6-Dimethylpiperidine		32
1-Ethylpiperidine	Affords efficient extraction of penicillin from rich acetate via the preparation of this salt	20
1-(*o*-Aminophenyl)-piperidine		79
2-Hydroxy-1-piperidinomethyl-napthalene	Sparingly soluble, therapeutic value	78
2-Methyl-1-(2-*N*-methylanilino-ethyl) piperidine	Sparingly soluble, repository action	81
N-(β-chlorophenethyl) piperidine	Water insoluble, non-toxic	12

Amine	Remarks on the Penicillin Salt	Ref.
1, 2-Diphenyl-2-(4-methylpiperidino) ethanol	Sparingly soluble repository action	81
1, 1-Diphenyl-3-(1-piperidyl) propane	Water insoluble, repository action	9, 27
1-Benzamido-1-phenyl-3-piperidino-propane		67
Aminopyrimidine		69
2-NH_2-pyrimidine		106a
2-Amino-4-methylpyrimidine		106a
2, 4-$H_2N(MeO)$ derivs. of pyrimidine		106a
4, 2, 6-$Me(H_2N)$-MeO derivs. of pyrimidine		106a
2, 4, 6-$H_2N(MeO)_2$ derivs. of pyrimidine	Repository action in hypodermic and oral use	106a
4, 6, 2-Me_2 (NH_2) derivs. of pyrimidine		106a
4, 2, 6-Me (H_2N)-EtO derivs. of pyrimidine		106a
4, 5, 2, 6-Me_2 (H_2N)-MeO derivs. of pyrimidine		106a
dl-1- [∝-(*p*-chlorophenyl) benzyl]-4-methylpiperazine	Oily suspensions give prolonged blood levels of penicillin	87
1, 2-Dibenzylpiperazine	Sparingly soluble, repository action	90
Melamine		14
$C_6H_5C_6H_5CH\ (CH_2)_2$-morpholine		27
4-[2-(3-Pyridyl) ethyl] morpholine	Sparingly soluble, repository action	81
9-Aminoacridine	Injectable suspension of the salt prepared	76
6-Chloro-9(4-diethylamino-1-methyl-butylamino)-2-methoxyacridine	Repository action	39
5-Aminoacridine		55
5-Amino-1-methylacridine		55
Atebrin	Solubility 15,000 u/cc. Suggested for purification of penicillin as the salt is soluble in chloroform	58
2-Ethoxy-6, 9-diaminoacridine		26
N-(2-dimethylaminopropyl)-phenothiazine	Oily suspension gives prolonged blood levels of penicillin	87
∝-(4-Quinolyl)-5-vinyl-2-quinuclidinemethanol		59
∝-(6-Hydroxy-2-quinolyl)-5-vinyl-2-quinuclidinemethanol		59
XVI. **Alkaloids**		
l-Ephedrine		32
dl-Desoxyephedrine		32
Quinine	Prolonged effective levels of penicillin in blood	59

Amine	Remarks on the Penicillin Salt	Ref.
Emetine	Useful for oral and external applications	35
XVII. Miscellaneous Amines		
N-isobutylbenzylamines nuclearly substituted in *m*- or *p*- positions with alkoxy groups of 1-4C atoms or phenoxy or benzyloxy groups		24
$PhCHRNH_2$ where R is alkyl, or alicyclic group		45
Ph_2CHNHR where R is alkyl, or aralkyl group		45
p-$RC_6H_4CHPhNH_2$ where R is alkyl, alicyclic, aryl or MeO group		45
Substituted alkylenediamines	Bronchodilatory and antihistamic action. Useful for separating penicillin from aqueous solutions as the salts are insoluble in water.	94
Substituted alkylenediamines such as (*i*) RN $(CH_2)_nNH_2$ (*ii*) RNH $(CH_2)_nNHR'$ (*iii*) RR″ $N(CH_2)_nNH_2$, where R, R′, R″ are aliphatic or aromatic or alicyclic or heterocyclic group		96
Mono- and di- substituted alkyleneamines RR′ NYNHR″ where R is substituted or unsubstituted aliphatic, aromatic, araliphatic, alicyclic or heterocyclic radical, R′ is H or R, R″ is H or when R′ is H then R″ is R, and Y is an alkylene radical with 2-12 C atoms	Suggested for use in separating penicillin from the broth by forming sparingly soluble salts.	92
R-NH-CH_2-CH_2-NH-R, where R is Me, Et, *N*-propyl, *N*-Bu, *N*-amyl, *N*-hexyl, *N*-heptyl, *N*-octyl, *N*-lauryl.	(*See* text)	104
$xCH(R)CH_2NR'_2$, where R and R′ =H or alkyl and x=(*i*) phenyl-2-furfuryl-methoxy or (*ii*) *p*-methoxy-2-pyridylamino or (*iii*) benzyl-pyridylamino or (*iv*) $PhCH_2NPh$ or (*v*) diphenylmethoxy or (*vi*) phenothiazinyl	Antihistaminic activity	88
Tetrasubstituted alkylenediamines		97
1, 4-Diamino-2-butene of the type $RNHCH_2CH:CHCH_2NHR$, where R is alkyl, alkenyl, aralkyl, hetero-cyclic-alkyl or alicyclic-alkyl group.	Effective blood levels of penicillin for over 96 hr.	1
$ROCH_2CH_2R'$ (R=*iso*bornyl, R′= piperidine or morpholine)	Sparingly soluble. Non-toxic	2
p-$H_2NC_6H_4COR$ (R=Ph, Me or Et)	Sparingly soluble. Non-toxic.	2
5-(Nitro-2-furfurylidine amino)-guanidine		65

Amine	Remarks on the Penicillin Salt	Ref.
1-(*p*-Chlorophenyl)-5-*iso*propylbiguanidine	Therapeutic advantages claimed	105
Guanylurea and derivatives	Sparingly soluble	105
N, N′-bis (*p, p′*-carbodiethylamine-ethoxyphenyl) urea		63
$PhCH_2NPhCH_2C(:NH)NH_2$	Oily suspension gives prolonged blood levels of penicillin	87
$PhNHCO_2CH_2CH_2NHR$, where R is (*i*) Pr, (*ii*) cyclohexyl, or (*iii*) morpholine	*N*-phenylurethans, $PhNHCO_2CH_2CH_2NHR$ in which R is an alkyl group are sparingly soluble in water. Injection of 3 lac units of the penicillin salt of the amine in which R is a butyl group gives therapeutically effective level of penicillin for 72 hr. This salt is non-irritant, does not produce necrosis and suggested for oral administration.	22
Derivatives of urethans: (*i*) 2-Butylaminoethyl-*N*-(4-ethoxyphenyl.) (*ii*) 2-Diethylaminoethyl-*N*-(4-hydroxyphenyl)		88
$XC{:}\,NCH_2CH_2NH$, where X is (*i*) benzyl or (*ii*) 1-naphthylmethyl or (*iii*) benzylphenylaminomethyl		50
Hexamethylenetetramine		
XVIII. Amides		
N, N′-bis(*p*-ethoxyphenyl) acetamide		77
$Et_2NCH_2CONPh_2$		11
$PrNHCH_2CONPh_2$		11
Propylaminoacetic acid diphenylamide		29
5-Nitro-2-furfurylacrylamide		65

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Note : Abbreviations used in the references :

BP=British patent ; CA=*Chemical Abstracts* ; Czech=Czechoslovakian patent ; GP=German patent ; Japan=Japanese patent ; US=United States patent.

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Research Reports

EFFECT OF PENICILLIN PRECURSORS ON THE GROWTH OF *PENICILLIUM CHRYSOGENUM*

ONE of the significant stages in the development of penicillin fermentation was the discovery by Moyer and Coghill[1] that phenylacetic acid stimulated the formation of benzylpenicillin (penicillin G). Kleiner and others[2] using phenylacetamide as a precursor with the strain "New Hybrid", found that it was a more efficient user of the precursor than the strain Wis. 51-20. Johnson[3] reported that phenylacetic acid (sodium salt) could be readily used by the mould as a carbon source. Work of Halliday and Arnstein,[4] and Deshpande and Ganapathi[5] indicates that buffer washed mycelium continues to form penicillin when suspended in a medium of phenylacetic acid. When it is remembered that phenylacetic acid is a plant growth regulating substance the question arises whether the precursor has any special function in relation to growth of *P. chrysogenum*.

The growth of a high yielding strain of *P. chrysogenum* (HA-3), developed in this laboratory, was studied in relation to the influence of phenylacetic acid, phenylacetamide and phenoxyacetic acid—the precursor for penicillin V.

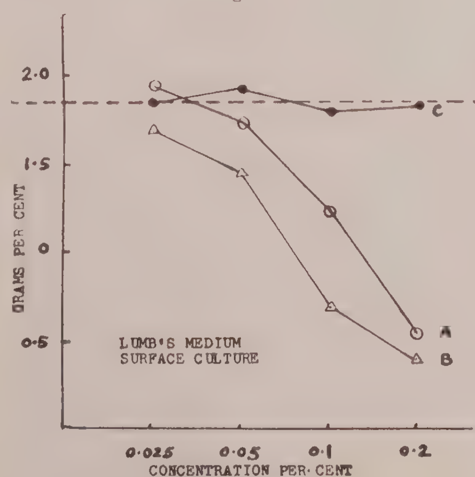
Materials and Methods

The strain HA-3 is a white spored derivative of Wis. Q-176. All the stocks were maintained in soil and transferred to slants. After five days, spores from slants were used to inoculate different media. Shaken cultures as well as liquid surface cultures were grown in 500 ml. Erlenmeyer flasks each containing 100 ml. of the medium. A rotary shaker with 280 r. p. m. was used for shaken cultures. For solid medium a portion of mycelial growth was transferred to the centre of a petri plate.

The types of media used were: (1) Production medium containing cornsteep liquor (6%), lactose (3.5%), $CaCO_3$ (0.48+) and minerals; (2) Lumb's medium; and (3) synthetic medium (chemically defined) according to Czapek-Dox formula. The initial pH in all cases was adjusted to 6.0 as, at lower pH ranges, the undissociated molecule of the precursors would be inhibitory to the mould.[6]

For surface culture both liquid and solid media were used. In the case of solid medium, diameter of the colonies was

Fig. 1.



– – – – – control.

measured after 10 days and there were ten replicates for each treatment. For surface liquid culture and for submerged cultures dry weight percentage of the filtered mycelium was taken as usual after acid washing to remove any unutilised

TABLE I.—PRECURSORS AND GROWTH OF *P. CHRYSOGENUM*

	Surface Culture Diameter in cm.				Surface Culture Dry wt. in gm.%				Submerged Culture Dry wt. in gm. %							
	Solid medium, synthetic				Liquid medium, Lumb's medium				Lumb's medium				Production medium, cornsteep-lactose			
Concentration %	0.025	0.05	0.10	0.20	0.025	0.05	0.10	0.20	0.025	0.05	0.10	0.20	0.025	0.05	0.10	0.20
Precursors: Phenylacetic acid (curve A) ..	2.67	2.18	1.81	1.61	1.93	1.76	1.26	0.55	1.97	1.68	1.53	1.47	2.21	2.08	2.0	2.17
Precursors: Phenylacetamide (curve B) ..	2.12	1.53	1.24	0.70	1.73	1.43	0.71	0.42	1.73	1.63	1.40	1.16	1.98	2.01	2.05	2.15
Precursors: Phenoxyacetic acid (curve C) ..	2.25	2.25	2.24	2.24	1.88	1.92	1.72	1.73	1.82	1.82	1.90	1.89	2.12	2.05	2.15	2.02
Control (without precursor)	2.24				1.84				1.83				2.14			

calcium carbonate. Dry weight per cent was determined after 8 days in the case of shaken cultures and after 15 days in the case of stationary cultures. There was no visible autolysis at the end of the period. Four concentrations of the precursors, 0.025%, 0.1%, and 0.2%, were used.

Fig. 2.

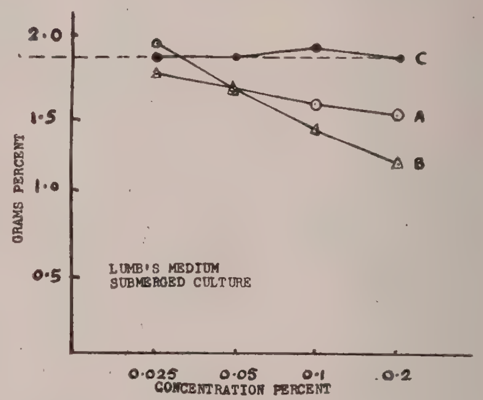
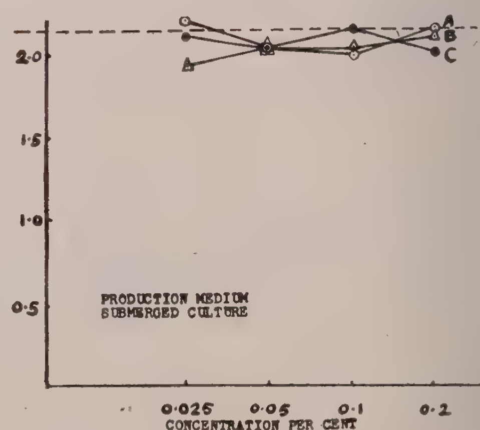


Fig. 3.



– – – – – control.

The results are given in Table I and figs. Readings of shaken cultures are a mean of 3 and those of surface cultures a mean of 10.

It is evident from the table and graphs that phenoxyacetic acid is non-inhibitory to the mould in all the concentrations and media. Phenylacetic acid and phenylacetamide seem inhibitory in surface cultures and only in Lumb's medium in submerged cultures. Of the two precursors the amide appears to be more inhibitory. It seems production medium might contain substances capable of counteracting the inhibitory effect of phenylacetic acid and amide in so far they affect the growth of the fungus.

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EFFECT OF THE METHOD OF PREPARATION OF CHEMICALLY DEFINED MEDIUM ON PENICILLIN YIELD WITH *PENICILLIUM CHRYSOGENUM*

SOLUTIONS of sugars when heated with amino acids or other nitrogenous compounds, inorganic acids or alkalis and certain inorganic salts produce brown to black colour and pronounced odour. The sugar colorants are apparently formed by the polymerisation of unstable fragmentary compounds produced from the sugars by dehydration and dealdolisation.[1] Ligett and Dietz[1] have reviewed the chemistry of colour formation during caramelisation. The colorant behaves like an indicator in that it changes colour with change in pH, but unlike the usual indicators, it ordinarily changes in the amount of colour rather than in the kind of colour. Carreras Leden and Pita Larraneta[2] have studied the effect of pH, inorganic salts and amino acids on the production of caramel during heating of various sugars. They found that the amount of colour produced increased with increasing pH. The absorption curve of the colour decreased in a regular manner from 350 mμ to 650 mμ. Hachisuka *et al*[3] studied the effect of caramels on the germination of spores of *B. subtilis.* They found that the rate of spore germination was highest when the medium contained caramel prepared from various sugars by heating. When intact glucose was used the rate of germination was found to be very low.

Normally in the preparation of chemically defined medium for growth of micro-organisms the sugars are sterilised separate from other constituents of the medium (NH_4-salts and essential inorganic salts) to avoid caramelisation of the sugar. This paper reports the effect of simultaneous sterilisation of the components of the chemically defined medium on growth and penicillin production by *P. chrysogenum.*

Materials and Methods

A commercial strain of *P. chrysogenum* HA-9 (a mutant obtained from a Russian strain) was used in these studies. Spores were obtained by sporulation on sterilised barley grains. The seed medium used as control (natural medium) contained (in g./1.): Sucrose 20, cornsteep liquor 31.6, $CaCO_3$ 3.2, $NaNO_3$ 2.8, $MgSO_4$ 0.068,

and KH_2PO_4 0.31. When synthetic medium (composition described in the course of experiment) was used, 10 ml. of salt mixture were added per 100 ml. of the medium. The salt mixture had the following composition (in g./l.): KH_2PO_4 30, $MgSO_4.7H_2O$ 2.5, $Fe(NH_4)_2SO_4.6H_2O$ 1.0, Na_2SO_4 5.0, $CaCl_2.2H_2O$ 0.5, $ZnSO_4.7H_2O$ 0.2, $MnSO_4.H_2O$ 0.2, $CuSO_4.5H_2O$ 0.05. A spore suspension (usually containing 10 to 20 million spores) was inoculated into 100 ml. of the seed medium. The seed flasks were incubated on a rotary shaker (250 r.p.m. describing a circle of 2 in. diameter) for 48 hr. at 25°. Ten ml. of the vegetative inoculum were used for seeding 100 ml. of the fermentation medium.

All fermentations were carried out in duplicate on the rotary shaker, at 25°, in 500 ml. Erlenmeyer flasks containing 100 ml. of the medium. The fermentation medium, unless stated otherwise, contained (in g./1.): Lactose 40, cornsteep liquor 10, peanut meal 30, Na_2SO_4 1.2, $MgSO_4.7H_2O$ 0.068, $CaCO_3$ 6, phenylacetic acid 1, and groundnut oil (arachis oil) 1 ml. The pH of the medium was adjusted when necessary to 6.8 after sterilisation. All media were sterilised for 30 min. at 15 lbs. pressure.

Analytical Procedure

Samples for analysis were withdrawn under aseptic conditions. The pH of the sample was determined with a glass electrode. The penicillin in the centrifuged broth was estimated by a modified iodometric method[4]. Ammonia nitrogen was determined by the method described by Gailey *et al*[5]. To determine the mycelial dry weight, the broth was filtered through a Buchner funnel and the mycelium washed with 0.1N HCl. The mycelium was then dried overnight at 90°.

Results and Discussion

Effect of composition of seed medium on penicillin yield: The results obtained when seed grown on different media was used to inoculate the fermentation medium are shown in Table I. When chemically defined

TABLE I: Effect of Composition of Seed Medium on Penicillin Production

Nitrogen Source		Seed Dry Wt. g/100 ml.	Penicillin units/ml.	Time of Maximum Yield (Hrs.)
Compound	Concentration %			
NH_4NO_3	0.8	1.03	2670	120
$(NH_4)_2SO_4$	1.3	1.10	2725	120
$(NH_4)_2HPO_2$	1.5	1.0	2570	144
Control *	—	1.0	2740	120

* The composition of the control seed medium is given under "Methods".

The synthetic seed media contained (per 100 ml.) $CaCO_3$, 1 g., sucrose, 3 g. ; and salt mixture, 10 ml. The sucrose was sterilised separate from the other constituents of the medium.

Ten ml. of vegetative seed inoculum was used to inoculate 100 ml. of the fermentation medium.

seed medium was used, the sugar was sterilised separate from the other constituents of the medium. It was found that the same amount of growth was obtained in all the seed media. The penicillin yields obtained were the same in all cases except when the synthetic seed medium contained ammonium phosphate as nitrogen source. Results were rather erratic when ammonium phosphate medium was used. Since best results were obtained with NH_4NO_3 and $(NH_4)_2SO_4$ further experiments were done with only these two nitrogen sources in synthetic seed medium.

Effect of pH on caramelisation of sugar during sterilisation and subsequent growth of seed inoculum: The effect of pH on caramelisation during autoclaving of sucrose or glucose in a medium containing NH_4NO_3 and salts was studied. The results are shown in Table II. It was visually observed that the reddish brown colour indicative of caramelisation was not developed when sucrose was used. With glucose caramelisation was noticeable at pH 6.0 and above. The

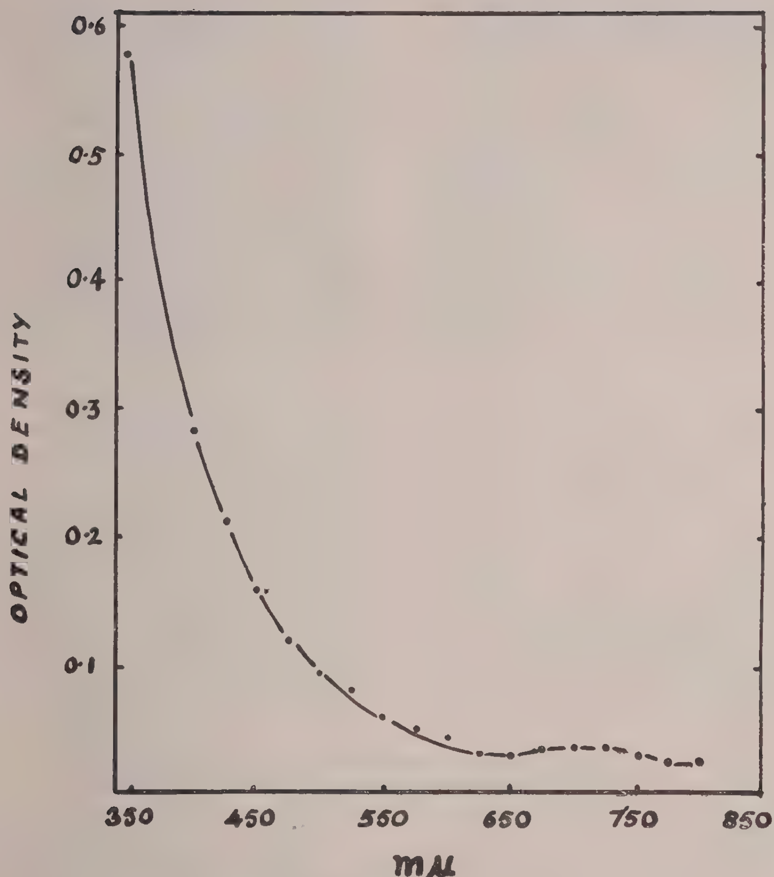

Fig. 1. Optical density of caramelised sugar at different wavelengths. Glucose was caramelised during autoclaving in a medium containing NH_4NO_3 and salts at pH 7.0.

absorption spectrum, in the visual region of the colour developed with glucose at pH 7.0 was measured (fig. 1). It was found that the absorption decreased in a regular manner from 350 mμ to 700 mμ. It has been pointed out[1] that the colorant changes colour with pH. However, unlike the usual indicators, it ordinarily changes in amount of colour rather than in kind of colour. Therefore, the optical density of all sugai solutions was measured at 375 mμ with water as the reference. It was found (Table II) that the optical density of the medium containing glucose at pH 7.0 was 10 times that at pH 5.0. The optical density of the medium, containing sucrose showed little change with pH. In order to determine the growth supporting ability of the caramelised sugar all media were adjusted to approximately pH 6.0 after sterilisation and then inoculated with spores. Good growth (Table 2) was obtained in all cases after 48 hours as compared to that in control seed medium.

Simultaneous sterilisation of seed medium components and penicillin yield: The results obtained with chemically defined seed

TABLE II: Effect of pH on Caramelisation of Sugar during sterilisation of Medium and subsequent growth of *P. chrysogenum*

Medium characteristics		Optical density at 375 mμ.	Seed dry wt.
Sugar	pH (before sterilisation)		g/100 ml.
Sucrose	5.2	0.20	0.90
Glucose	5.2	0.15	0.88
Sucrose	6.0	0.21	0.85
Glucose	6.0	0.49	0.90
Sucrose	7.0	0.22	0.88
Glucose	7.0	1.60	0.81
Sucrose	7.6	0.25	0.85
Glucose	7.6	2.04	0.89
Control*	5.5	—	0.95

*The composition of the natural seed medium used as control is given under "Methods".

All other media contained (per 100 ml.); Sugar, 3 g.; NH_4NO_3, 0.8 g.; and salts, 10 ml. The pH of the synthetic seed media was adjusted to 6.0 prior to inoculation.

medium in which all the components were sterilised together and the pH of the medium was not adjusted after sterilisation, prior to inoculation, are shown in Table III. It was found that when the seed medium contained sucrose, NH_4NO_3 and salts good mycelial growth was obtained and during growth the pH was maintained at about 6.0. When a seed medium containing sucrose, $(NH_4)_2SO_4$ and salts was used the utilisation of NH_3-N for the growth of the mould lowered the pH to 1.6 which was unsuitable for growth. This indicated that in the medium containing sucrose, NH_4NO_3 and salts the ammonia-nitrogen was utilised at the same rate as the nitrate-nitrogen thus maintaining the pH in a favourable region. This assumption was substantiated by chemical analysis of the sucrose-NH_4NO_3-salts medium (Table IV). The mycelial nitrogen was calculated on the basis of 7 per cent nitrogen in the mycelium[6]. It may be seen that the decrease of ammonia nitrogen in the broth accounted for about half of the mycelial

TABLE III: Penicillin Production with Seed Grown on Synthetic Medium (Medium Components Sterilised Together)

Nitrogen Source		Buffering Compound		pH			Seed Dry wt. gms./100 ml.	Penicillin units/ml. (in 120 hrs.)
	Conc. %		Conc. %	Before Sterilisation	After Sterilisation	After 48 hrs.		
Control* ..	—	—	—	5.5	5.5	7.2	1.02	2560
NH_4NO_3 ..	0.8	—	—	6.0	6.25	6.0	0.80	2650
NH_4NO_3 ..	0.8	$CaCO_3$	0.4	5.4	6.0	5.7	1.16	2350
NH_4NO_3 ..	0.8	K-acetate	0.4	6.0	6.1	5.6	1.03	2500
$(NH_4)_2SO_4$ ..	1.3	—	—	6.0	6.0	1.6	Nil	—
$(NH_4)_2SO_4$ ..	1.3	$CaCO_3$	1.0	5.5	8.4	9.4	Nil	—
$(NH_4)_2SO_4$ ..	1.3	$NaNO_3$	1.0	6.0	6.1	5.6	0.82	—
$(NH_4)_2SO_4$ ..	1.3	K-acetate	0.6	6.0	6.1	2.5	0.50	—

* The composition of the natural seed medium is given under "Methods" and was taken as control. All the synthetic media (seed) contained (per 100 ml.). Sucrose, 3 g; and salts mixture, 10 ml. Ten ml. of the seed were used to inoculate 100 ml. of the fermentation medium.

TABLE IV: Nitrogen Utilisation during Growth of *P. chrysogenum* in Sucrose-NH_4NO_3 Seed Medium

Hours of growth	pH	NH_3-N in broth mg./ml.	NH_3-N utilised mg./ml.	Mycelial dry wt. mg./ml.	Mycelial nitrogen mg./ml.
0 ..	6.0	1.46	—	—	—
24 ..	—	1.44	0.02	—	—
48 ..	6.05	1.20	0.26	8.0	0.56

Composition of seed medium (in g./100ml.): Sucrose 3; NH_4NO_3, 0.8; salt mixture 10 ml.; all components of medium were sterilised together.

nitrogen, indicating that the rest of the mycelial nitrogen was deiived from nitrate-nitrogen.

When a medium containing sucrose, $(NH_4)_2$ SO_4 and $CaCO_3$ was used the pH after sterilisation rose to a level unsuitable for growth. In the medium containing sucrose, $(NH_4)_2$ SO_4 and potassium acetate, the pH during growth was lowered to 2.5. Therefore poor growth was obtained with this medium also. With all other media a

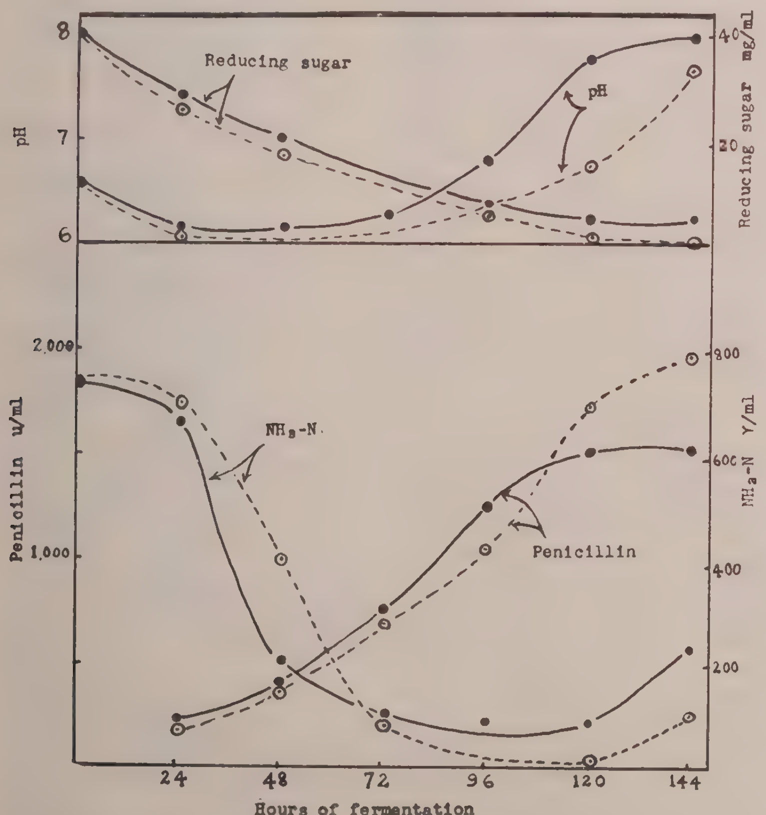

Fig. 2. Effect of caramelisation of sugar on penicillin production in synthetic medium.

●——● Sugar in the medium was sterilised together with the other components, thereby forming caramelised sugar.

o- - - -o Control fermentation. Sugar in the medium was sterilised separate from the other components of the medium.

pH more or less favourable for growth was obtained after sterilisation.

The penicillin yields obtained with seeds grown in NH_4NO_3 medium compared favourably with those obtained with the seed grown on control medium containing cornsteep liquor, sucrose and $CaCO_3$ (Table III).

Penicillin production with synthetic seed and fermentation media containing caramelised sugar: The results obtained with synthetic seed and fermentation media of Jarvis and Johnson[7] are shown in fig. 2. The media components were sterilised together. The results are compared with those obtained with a control fermentation in which the sugar was sterilised separate from the other components of the medium. It will be seen that the rate of utilisation of the reducing sugar was the same in both the caramelised and uncaramelised forms. The utilisation of ammonia nitrogen was also the same, indicating that the same amount of growth was obtained in both cases. Penicillin yield obtained with the caramelised medium compared favourably with the control.

SUMMARY

The effect of caramelisation of glucose and sucrose during simultaneous sterilisation with inorganic constituents of chemically defined media was studied with reference to growth of *P. chrysogenum* and penicillin biosynthesis. Sucrose was caramelised to a much lesser extent than glucose. Caramelisation did not affect the growth and penicillin production by the mould.

In a synthetic seed medium containing sucrose, NH_4NO_3 and salts at pH 6.0, the ammonia-N, was utilised at the same rate as the nitrate-N, thus maintaining the pH constant during germination and growth of mould mycelia.

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Physico-chemical Data on Antibiotics

I. ANTIBIOTICS PRODUCED BY FUNGI, BACTERIA AND LICHENS

3. Optical Rotation.

AVAILABLE data on the optical rotation of antibiotics produced by bacteria, fungi and lichens, are given in the table below. The compounds listed in both the earlier papers[1] of this series are covered.

A. NEELAMEGHAN

Library,
Hindustan Antibiotics Ltd.,
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Specific rotation $(\alpha)_D$	Solvent	Concentration	Temp. (°C)	S. No. of Antibiotic[1]
		DEXTROROTATORY		
3.0	95% EtOH	0.17	20	182
4.4	$CHCl_3$	0.8	23	67
5.9	$CHCl_3$	2.0	23	23
7.5	EtOH	1.0	20	122
8.19	C_5H_5N	5.1	20	164
11.9	Absol. EtOH		20	79
13.4	EtOH	0.85	24	51
15±2	$CHCl_3$	1.0	24	44
18.5	1*N* NaOH	1.05	24	51
20.0	Absol.EtOH	3.0	24	142
28.0				166
36.0	EtOH	1.0		52
44.0	$CHCl_3$	1.0	18	121
54.0 (approx.)	C_6H_6	0.2	20	171
58.0	$CHCl_3$	1.0		57
74.3	Phosphate buffer, pH 7			7
82.0	$CHCl_3$		24	65
103.0	Water	0.9	20	88a
105.0	96% EtOH		22	161
116±1	EtOH		21	151
149.0	$CHCl_3$	0.8	20	93
165.0	2%$NaHCO_3$		22	161
175.0	$CNCl_3$		20	92
176.0	C_3H_6O	1.4	25	75
179·0	$CHCl_3$	0·8	20	93
187·0	Water	0.6	20	66
202.0	EtOH	1.2		39
203.0	$CHCl_3$		20	96
215.0	Water	1.06		87
258.0	Water	0.43	25	90
267.0	Water	0.525		86
270.0	EtOH	0.44	23	41
276 to 316	Water	0.82	20-25	63
301.0	Water	2.0	25	84
305.0	Water	0.821	25	84
337·0	C_6H_6O	1.0	21	97
350·0	Alcohol		17	39
360.0	$CHCl_3$	1.0	22	83
370.0	$CHCl_3$	2.0	17	97
380.0	Alcohol		17	41
390.0	C_3H_6O	1.0	19	98
445 to 515	$CHCl_3$	2.0-3.19	20	107
500.0	$CHCl_3$	1.0	21	133
605.0	Dioxane			162
		LAEVOROTATORY		
2.8	HAc	5.0 in 0.01	25	180
3.6	HAc	5.0 in 0.01	25	178
4.8	Water	0.012	32	223
11.4	Alcohol		20	45
16.0	$CHCl_3$	1.0	22	7
18.9			20	46
18.9	MeOH	1.0	26	190
20.0	$CHCl_3$	0.59	23	127
22.0	0.1*N* HCl	0.8	25	31
24.0	$CHCl_3$	5.0	25	158
26.6	MeOH	0.25	25	158
28.6	50%CH_3OH-50% C_6H_6	1.0		7
29 to 35	Acetic acid		23	296
36.8				194
37.4				194
37.4	EtOH	1.15	18	58
37.7				194
42 to 48	Water, pH 7			28
43.1	Absol.EtOH	0.92	23	58
47.0	Water		20	35
47.5	Water	2.0	20	31
48.6	Water	1.0	25	31
50.7	$CHCl_3$		20	117
60.1	Water	2.094	25	170
61.0	0.1*N* NaOH	0.8	25	31
61.6	Water	1.25	25	170
83.2±2	EtOH	1.0	21	149
84.0	CHCl3	3.0		167
85.1	75% EtOH	2.33	25	174
90 to 92	$CHCl_3$	about 1.0		148
92±1.6	EtOH	1.2	19	160
93.3	Isopropyl alcohol		20	173

1. *Hindustan Antibiotics Bulletin* **2**, 18-38, 54-65, 1959.

Specific rotation $(\alpha)_D$	Solvent	Concentration	Temp. (°C)	S. No. of Antibiotic[1]
101.0				181
101±2	EtOH	1.0	19	155
102.0	95% alcohol	1.0	25	181
103±2	EtOH	1.0	18	159
106 to 108	$CHCl_3$	0.63 to 1.2		143
108·15	$CHCl_3$		12	29
111.0	50% EtOH	1.37	25	176
113.0	$CHCl_3$	3.1	23	165
117.0	$CHCl_3$	2.6	23	165
124.0	$CHCl_3$	1.0	25	165
126.0	Absol.EtOH		20	77
133.0			13.5	23
138.0				23
162.2			20	229
165.0	Absol.EtOH		20	78
168.3			29	229
182.0	$CHCl_3$	0.688	19	141
197.0	$CHCl_3$	0.6	19	73
213.4	$CHCl_3$		20	116
217.0	$CHCl_3$	0.8688	19	141
222.0	$CHCl_3$	1.0	19	117
239 to 256	$CHCl_3$			56
290±10	EtOH	0.078	25	56
292.0	EtOH	1.5	18	175
295.0	EtOH	1.5	20	175
308.9	EtOH		18	256
445 to 478	$CHCl_3$	2.0-3.08	20	107

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THE Pfizer name today calls to mind antibiotics, fermentation chemistry, pharmaceuticals and chemicals for medicine, agriculture and industry. It signifies an organization that does business in more than 100 countries around the globe.

But back in 1849 the name was new to America — a freshly-chiseled inscription in the slab masonry over the portals of a modest brick building in the Williamsburgh section of Brooklyn.

Germany's political upheaval in 1848 created a great wave of immigration across the Atlantic. From Ludwigsburg in the Kingdom of Wurttemberg came two young men, Charles Pfizer and Charles Erhart. Twenty-four-year-old Pfizer had already served his apprenticeship in a chemist's shop after a public school education. Erhart, a distant relative and close friend, was trained as a confectioner. Within a year they determined on a partnership, bought the small building in Brooklyn, and set up shop as manufacturing chemists.

The company soon began the manufacture of fine chemicals for the pharmaceutical and food industries — a business which still accounts for about 20 per cent of Pfizer's total sales. For the first 100 years, Pfizer sold its chemicals mostly in bulk to other manufacturers, serving first the infant pharmaceutical industry and later branching out into ingredients for food processors.

In 1862 Pfizer became the first American producer of tartaric acid and cream of tartar. However, it was Pfizer's early concentration on another of its products that set in motion the chain of events which ultimately brought the company to its present-day position as a leading chemical research organization. Searching for a new method of making citric acid (until then produced from the culls of citrus fruits) Pfizer chemists in 1917 developed a method for producing the chemical by fermentation of molasses.

The knowledge gained at that time and during the years that followed (when Pfizer scientists successfully applied the fermentation process to the manufacture of such other organic chemicals as gluconic acid, sorbose and riboflavin) gave company researchers the background that made possible the first commercial production of penicillin and the subsequent development of such vital "broad-spectrum" antibiotics as *Terramycin* and *Synermycin*.

A major turning point in Pfizer's history came in 1941, when a delegation of British scientists, headed by Sir Howard Florey, came to the United States to consult with the U.S. government on the problem of developing methods for mass production of penicillin. Because of their experience with molds and fermentation, Pfizer chemists were able to work out the complicated procedures and to build the first mass production penicillin plant. By the end of the war, the company was producing more than 50 per cent of the world's penicillin.

From these beginnings, company research made possible new and more potent drugs, including "broad-spectrum" antibiotics. *Terramycin*, Pfizer's first "broad-spectrum" drug is effective in combating more than a hundred different diseases.

Today, Pfizer manufactures a wide variety of products in addition to antibiotics. Some of the more important are vitamins and steroids, several of which have proven

* Courtesy: Chas. Pfizer & Co., New York, and Dumex Private Ltd., Bombay.

valuable in the treatment of arthritis and other previously intractable inflammations. *Viadril*, the first steroid ever developed for use as an anaesthetic, is a product of Pfizer research. Also the company produces such antitubercular agents as *Viocin* and *Streptohydrazid*, and a variety of pharmaceutical specialities.

But antibiotics and other drugs, no matter how spectacular their life-saving qualities, are of no value unless they are available when and where they are needed. For Pfizer, as for other pharmaceutical companies, the problems of making them available to patients in Hong Kong and Havana, Madras and Marseilles involve matters as complex in their way as the laboratory procedures that go into making them.

One of the most striking characteristics of Pfizer has been the phenomenal growth of its international operations. As recently as the beginning of 1951, there was not a single Pfizer product being produced or packaged outside of the United States. By contrast, there are today more than 8475 men and women of 61 different countries who are employees of Pfizer outside of the United States, and Pfizer products are being manufactured in 21 countries around the world.

When a product is manufactured locally, production and quality requirements must be maintained in accordance with the drug laws of the country and in keeping with Pfizer's own standards. Vials, bottles and capsules must be made available on a satisfactory schedule. Power, raw material and water shortages frequently plague Pfizer plant managers and those of the company's suppliers, while in some parts of the world excessive heat and humidity pose unique production, packaging, shipping and storage problems. In addition, there are extensive programs of local clinical investigation. Because Pfizer is in business in more than 100 different markets, it must deal, globally, with 100 different languages or dialects — and a great variety of regulations pertaining to health and new product registration.

In the face of these problems, Pfizer's growth over the past years may be considered particularly unusual. Pfizer's one simple concept is basic to the entire system: each country can best be served if it is considered as an individual domestic market rather than a segment of an international pattern of export trade.

Underlying all of Pfizer's international activities is its policy of decentralization, of "putting down roots overseas" as Mr. John J. Powers, senior vice-president of the parent company and chairman of the board of Pfizer International, describes it. These policies follow from his strong conviction that the company must become a citizen — not merely a tourist or interested solely in making quick profits — in the countries in which it does business. If Pfizer is to do business in a country, he feels, it must become part of the national industrial community and must contribute to the country's economic, social and scientific progress.

Pfizer's products are manufactured today not just in the United States, but in 20 other countries. These include France and Japan — where there are partnership arrangements with local companies — as well as England, Belgium, Brazil, Canada, the Philippines, Italy, Chile, Turkey, Sweden, Germany, Spain, Mexico, Australia, Argentina, Pakistan, India, and Ceylon. In each of these countries, and in others where there are Pfizer sales branches and subsidiaries, local autonomy is the basic principle. Marketing policy is determined by local managers and the emphasis is on hiring of local personnel, from top to bottom. In 1951, when Pfizer started operations in India, an Indian, now one of the Directors of Dumex Private Limited, was posted as Technical Representative to help the then Distributors. In 1954 distribution work was taken over by

Ravison Pharmaceuticals Private Limited who carried on till 1958 when Pfizer, realising the need for local manufacture in accordance with their world-wide policy, associated themselves with Dumex Private Limited which had already established extensive connections in the field of pharmaceutical manufacture.

If international trade is considered the muscle and sinews of Pfizer abroad, research is most surely its lifeblood. Research to Pfizer means many things, some of them going far beyond the popular concept of the laboratory. During 1957, Pfizer spent more than $ 10 million on research — on both pure research that may have no immediate practical value but serves the important purpose of extending the frontiers of scientific knowledge, and on applied research, which has the immediate goal of providing therapeutic agents for many a man's ills.

In the United States, one important centre of Pfizer research is located at Maywood, New Jersey, where work is carried on on a variety of still unconquered diseases. One of these is cancer, for which Pfizer scientists are engaged in the search for more effective chemotherapeutic agents. Pfizer's work in the cancer field has received recognition from the U.S. government's National Institutes of Health, which allotted the company government funds for screening possible anti-cancer compounds. More than 30,000 screening tests of antibiotic-like broths, botanical extracts and synthetic ehcmicals are now performed annually, exclusive of work done in co-operation with other laboratories engaged in cancer research. At the same time, additional studies are being undertaken to develop new methods of screening substances for possible anti-cancer activity.

Pfizer scientists are working, too, with radio-active isotopes — seeking to determine why and how antibiotics exert their therapeutic effect on the human body. From studies of this kind should come new and more potent — and perhaps, even "tailor-made" drugs to help cure mankind's ills.

Major research activity is also conducted by Pfizer at its 700-acre Agricultural Research Center in the midwestern United States. Here, research on animal feed supplements and veterinary products has shown that it is possible to speed up the growth of livestock and poultry, and increase the amount of milk and eggs produced for each pound of feed. These advances, which have real significance in the economics of agriculture, have been brought about by adding minute amounts of antibiotics to animal feeds. The lower production costs that result from the substantial savings and shortened feeding periods promise lower food prices for the consumer today — and, ultimately, more food for the world's fast-growing population.

Because of Pfizer's world-wide operations, the benefits of Pfizer research carried on in the United States are quickly made available in all countries of the globe. But Pfizer research is by no means limited to the United States. The scope of research activities abroad is expanding rapidly. Supported by grants-in-aid and fellowships, physicians and scientists of universities, hospitals and government agencies in many countries have worked closely with Pfizer scientists in a variety of studies. One series of Pfizer sponsored research projects that may hold promise of combating some of the world's nutritional problems, grew out of the previously-mentioned finding that livestock grow faster and better when given feeds containing small quantities of *Terramycin*. With this information and some imaginative speculation, studies were begun on the potentials of antibiotics for correcting growth failure in children. The results of these studies, carried on in Italy, Guatemala and Haiti, give some preliminary indications that antibiotics may help pro-

vide part of the answer to the protein deficiencies in many national diets.

Research is also spurred by Pfizer awards and by fellowships for advanced study by graduates of colleges of medicine and pharmacy. Pfizer companies in Australia, the Philippines, Japan, Portugal, England and other countries are sponsoring such programs. Even if nothing so spectacular results from these efforts, the company will still have made a major contribution to medicine and science by helping doctors and chemists to perfect themselves.

Research has brought about almost unbelievable developments in the past twenty years. Indeed, 70 per cent of the prescriptions written today in the United States are for products that did not even exist less than a generation ago. What will happen in the next twenty years? An antibiotic as successful against the viral diseases as today's are against bacterial infection? Therapeutic aids for one or more of the chronic degenerative diseases — heart disease, or perhaps, cancer — that today are becoming increasingly prevalent? Certainly there are many areas where research must still seek the answers needed to reduce human suffering and save human lives. In the search for these answers, the Pfizer company can be expected to maintain its present leading role.

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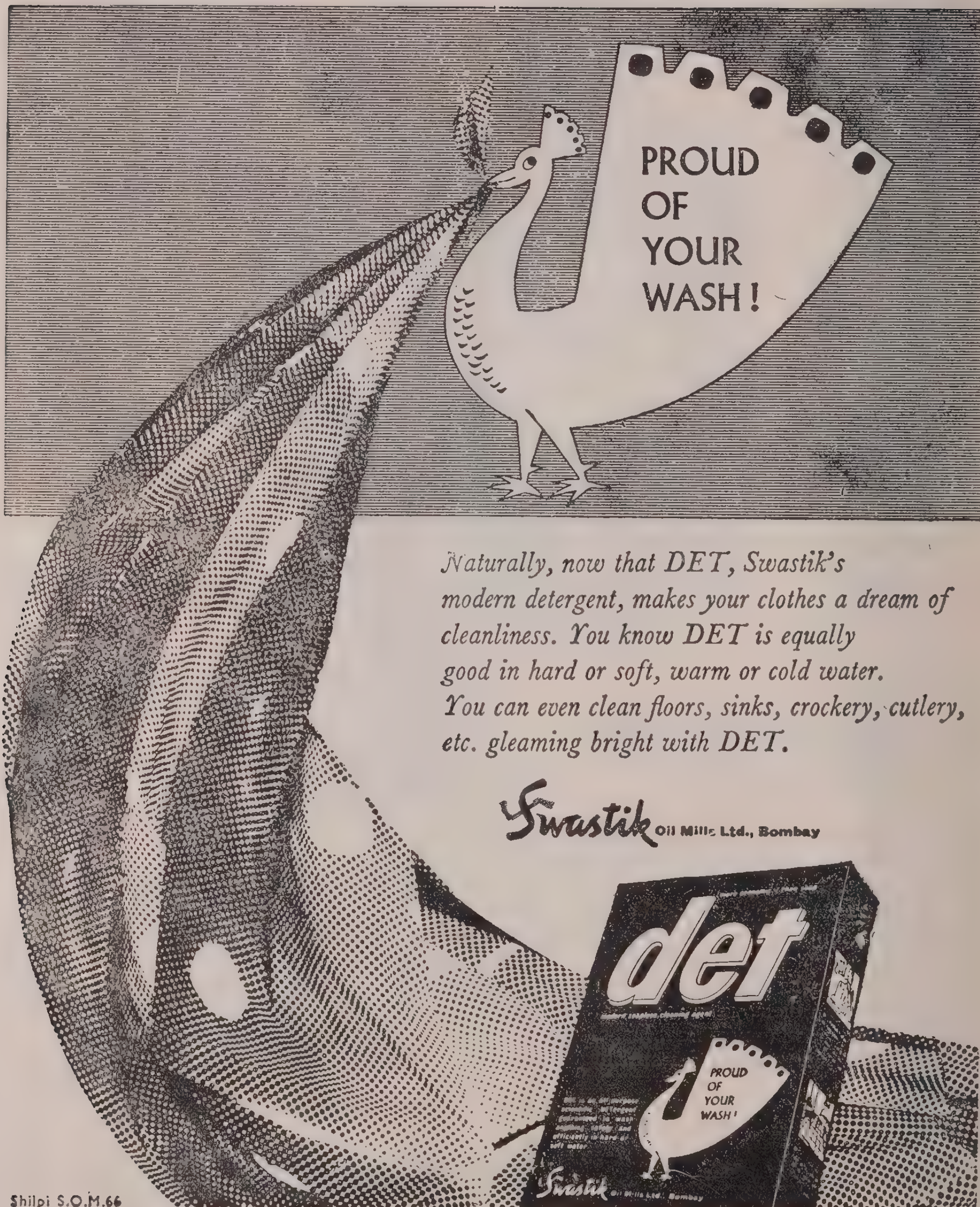
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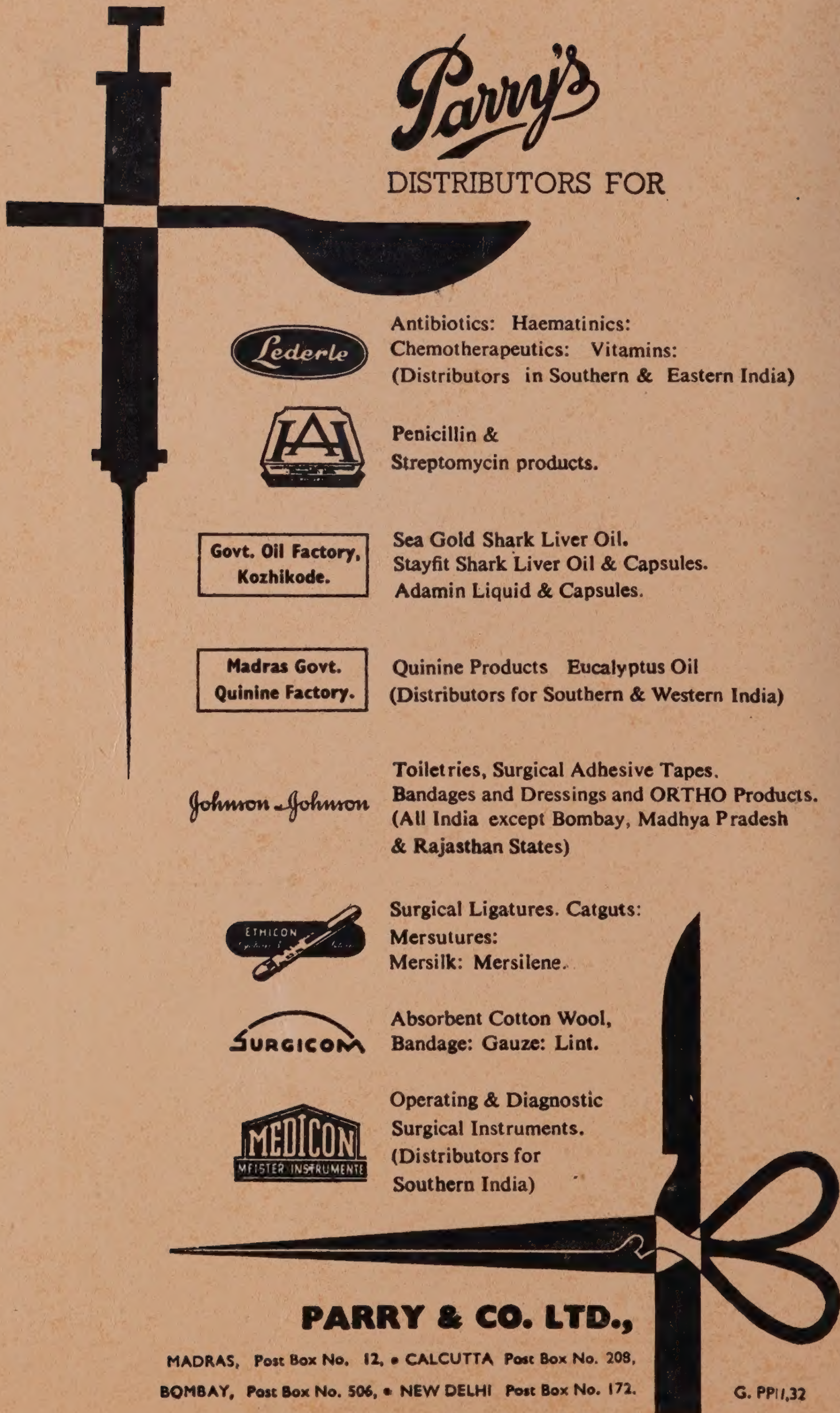
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